7-10 novembre 2013, Bari

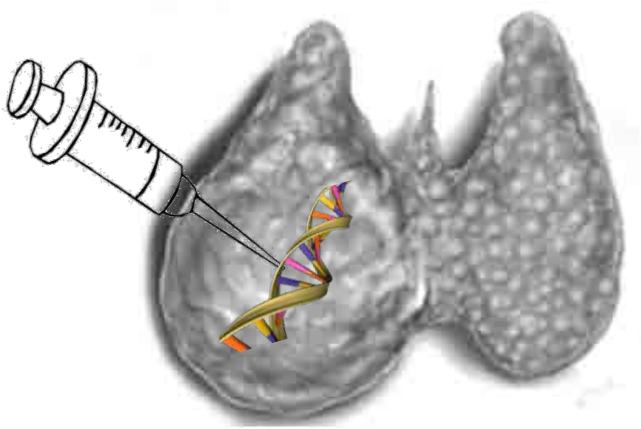
12° Congresso Nazionale AME
6th Joint Meeting with AACE

Update in Endocrinologia Clinica

## Symposium 13

Molecular markers in thyroid cancer: current role in clinical practice

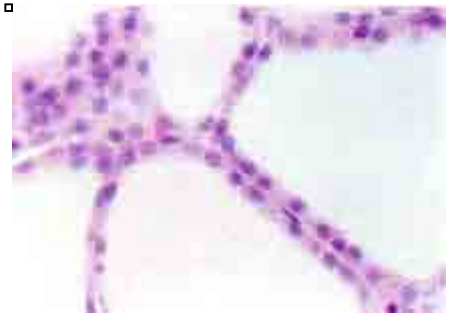
# BRAF in the diagnostic evaluation of thyroid nodules



*Laura Fugazzola*

*University of Milan, Italy*

UNIVERSITÀ DEGLI STUDI DI MILANO



**Follicular cell**

**Papillary carcinoma**

**BRAF**
***ret*/PTC**
***ras***
***met***

***P53***

Poorly differentiated carcinoma

***P53***

Anaplastic carcinoma

***P53***

***PAX8-PPARg***

***ras***
***PAX8-PPARg***

Follicular carcinoma

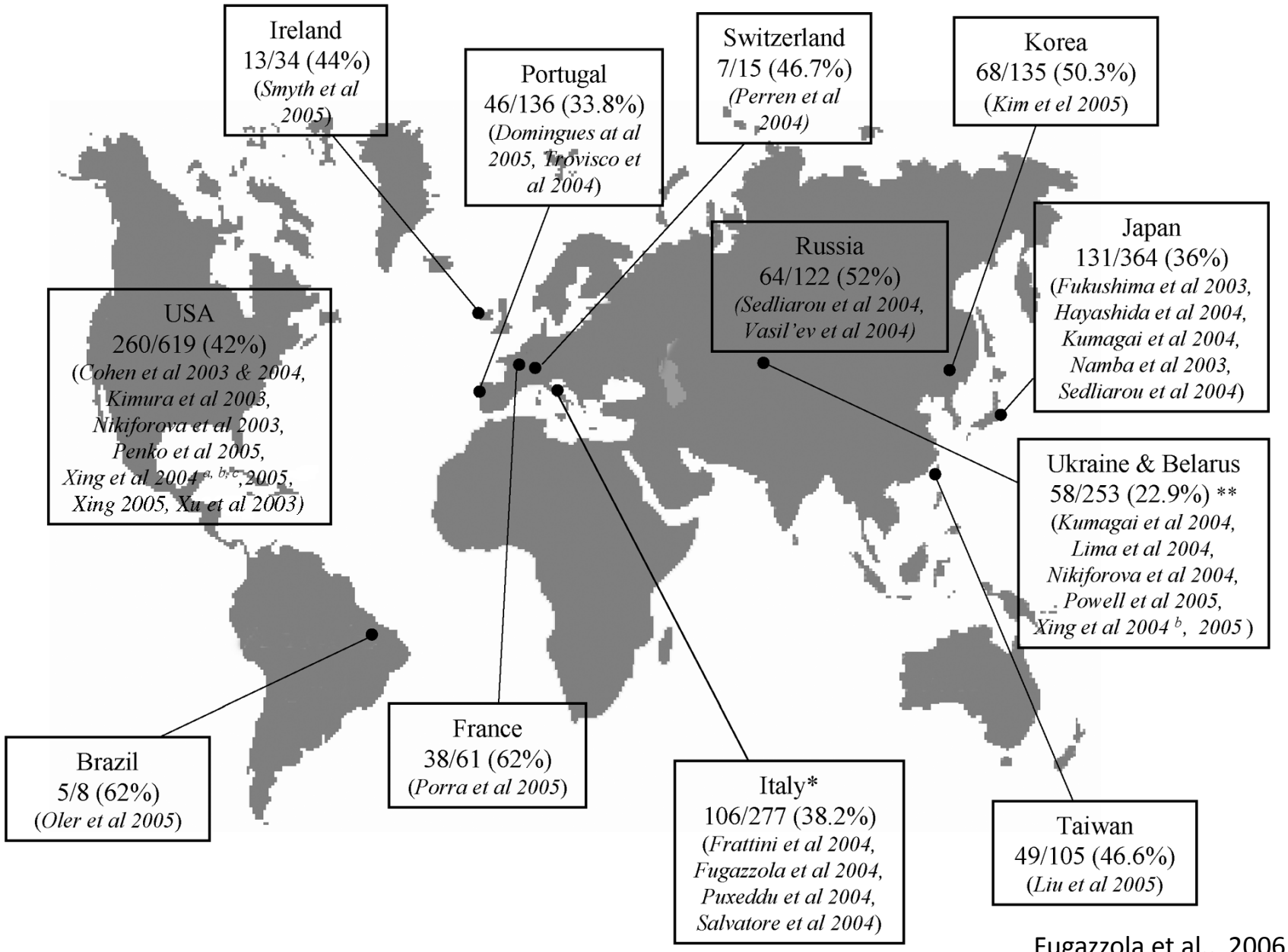
***ras***

***TSH-R***

Adenoma without autonomy

Adenoma with autonomy

# BRAF prevalence




Fugazzola et al., 2006

**Table 1.** Demographic Characteristics, *BRAF* V600E Mutation, and Follow-up Time of Patients by Medical Center and Country

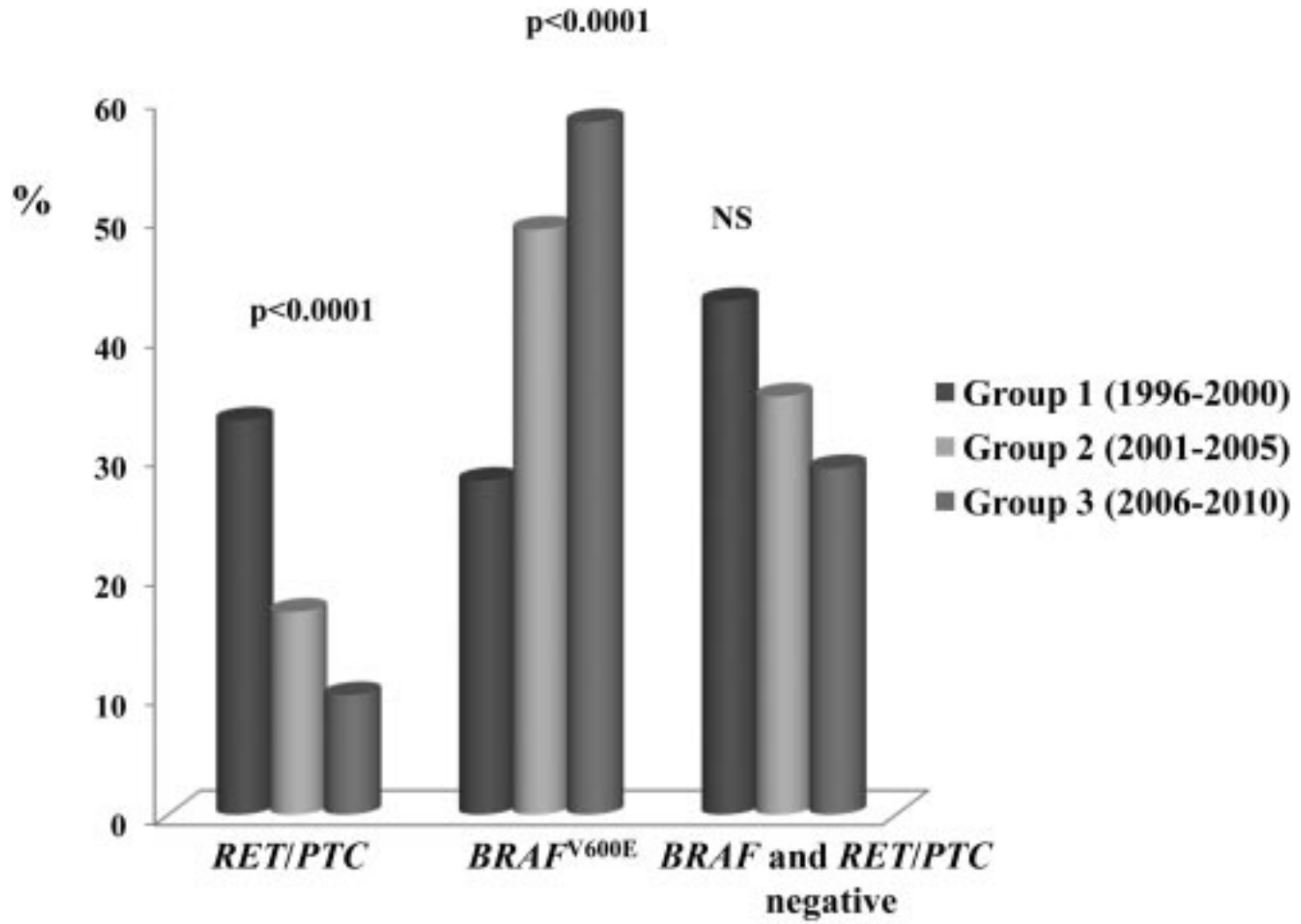
| | No. of Patients | Age at Diagnosis, Median (IQR), y | Male, No. (%) | *BRAF* V600E Mutation, No. (%) | PTC-Related Deaths, No. (%) | | | Follow-up, Median (IQR), mo | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | All | *BRAF* V600E–Positive | *BRAF* V600E–Negative | All Patients | Survivors |
| By medical center | | | | | | | | | |
| Johns Hopkins Hospital | 387 | 45 (35-57) | 101 (26.1) | 151 (39.0) | 8 (2.1) | 8 (5.3) | 0 | 12 (1-30) | 12 (1-28) |
| University of Pittsburgh | 169 | 52 (38-63) | 42 (24.8) | 101 (59.8) | 1 (0.6) | 1 (1.0) | 0 | 19 (11-26) | 19 (11-26) |
| Memorial Sloan-Kettering Cancer Center | 135 | 50 (35-63) | 44 (32.6) | 64 (47.4) | 11 (8.2) | 10 (15.6) | 1 (1.4) | 96 (1-144) | 90 (1-144) |
| University of Pisa | 189 | 38 (28-51) | 47 (24.9) | 65 (34.4) | 9 (4.8) | 6 (9.2) | 3 (2.4) | 72 (24-180) | 84 (24-180) |
| University of Perugia | 117 | 49 (37-59) | 32 (27.4) | 76 (65.0) | 5 (4.3) | 2 (2.6) | 3 (7.3) | 22 (6-39) | 22 (6-40) |
| University of Milan | 110 | 42 (34-55) | 24 (21.8) | 38 (34.6) | 1 (0.9) | 0 | 1 (1.4) | 48 (24-64) | 48 (24-64) |
| University of Padua | 135 | 48 (39-57) | 32 (23.7) | 87 (64.4) | 1 (0.7) | 1 (1.2) | 0 | 26 (22-30) | 26 (22-30) |
| Kanagawa Cancer Center | 49 | 55 (41-65) | 16 (32.6) | 33 (67.4) | 9 (18.4) | 7 (21.2) | 2 (12.5) | 68 (31-78) | 65 (33-76) |
| Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology | 99 | 49 (33-59) | 10 (10.1) | 42 (42.4) | 1 (1.0) | 1 (2.4) | 0 | 48 (42-53) | 48 (43-53) |
| Griffith Medical School | 76 | 40 (34-56) | 20 (26.3) | 34 (44.7) | 0 | 0 | 0 | 42 (4-82) | 42 (4-82) |
| University of Sydney | 95 | 44 (34-59) | 20 (21.0) | 55 (57.9) | 5 (5.3) | 5 (9.1) | 0 | 103 (63-135) | 104 (64-137) |
| Hospital La Paz, Health Research Institute | 66 | 42 (32-54) | 11 (16.7) | 28 (42.4) | 2 (3.0) | 1 (3.6) | 1 (2.6) | 41 (30-57) | 42 (30-57) |
| Institute of Endocrinology, Prague | 222 | 47 (31-60) | 39 (17.6) | 71 (32.0) | 3 (1.4) | 3 (4.2) | 0 | 50 (30-85) | 50 (30-85) |
| By country | | | | | | | | | |
| United States | 691 | 47 (36-59) | 187 (27.1) | 316 (45.7) | 20 (2.9) | 19 (6.0) | 1 (0.3) | 17 (2-36) | 16 (2-32) |
| Italy | 551 | 44 (34-56) | 135 (24.5) | 266 (48.3) | 16 (2.9) | 9 (3.4) | 7 (2.5) | 33 (20-70) | 34 (20-72) |
| Japan | 49 | 55 (41-65) | 16 (32.6) | 33 (67.4) | 9 (18.4) | 7 (21.2) | 2 (12.5) | 68 (31-78) | 65 (33-76) |
| Poland | 99 | 49 (33-59) | 10 (10.1) | 42 (42.4) | 1 (1.0) | 1 (2.4) | 0 | 48 (42-53) | 48 (43-53) |
| Australia | 171 | 43 (34-57) | 40 (23.4) | 89 (52.0) | 5 (2.9) | 5 (5.6) | 0 | 75 (32-118) | 76 (33-118) |
| Spain | 66 | 42 (32-54) | 11 (16.7) | 28 (42.4) | 2 (3.0) | 1 (3.6) | 1 (2.6) | 41 (30-57) | 42 (30-57) |
| Czech Republic | 222 | 47 (31-60) | 39 (17.6) | 71 (32.0) | 3 (1.4) | 3 (4.2) | 0 | 50 (30-85) | 50 (30-85) |
| Overall | 1849 | 46 (34-58) | 438 (23.7) | 845 (45.7) | 56 (3.0) | 45 (5.3) | 11 (1.1) | 33 (13-67) | 33 (13-65) |

Abbreviation: IQR, interquartile range.

Xing et al., JAMA 2013

## Modifications in the Papillary Thyroid Cancer Gene Profile Over the Last 15 Years

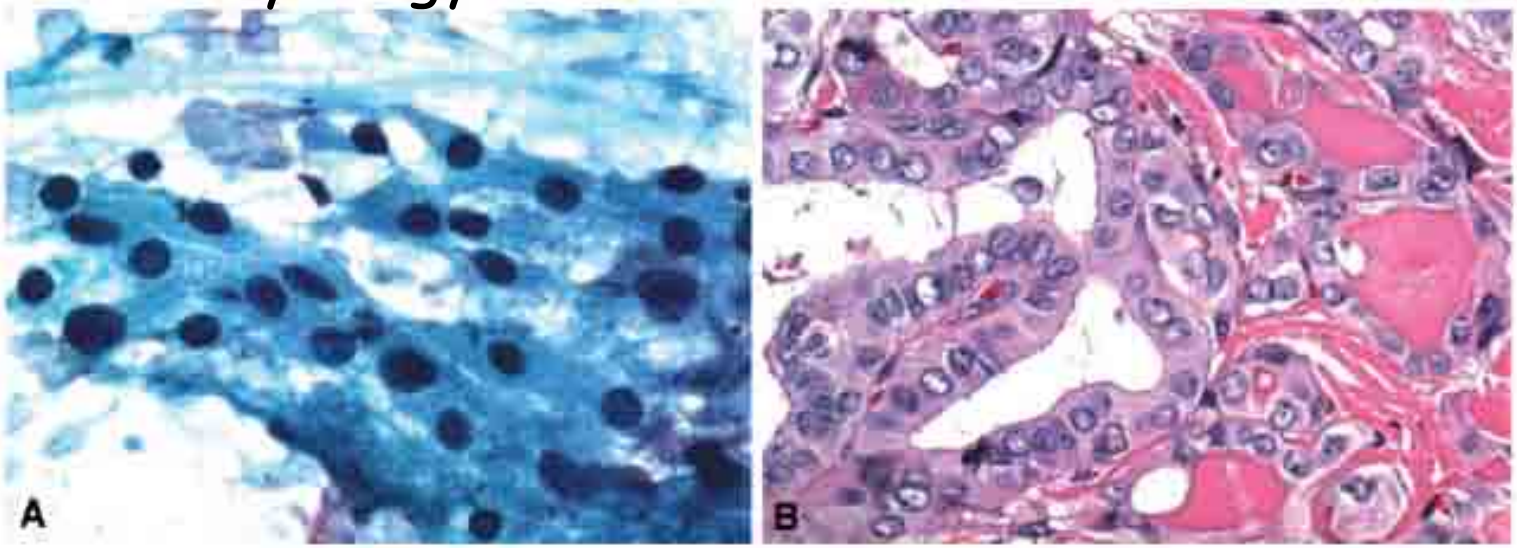
Cristina Romei,* Laura Fugazzola,* Efisio Puxeddu, Francesco Frasca, David Viola, Manna Muzza, Sonia Moretti, Maria Luisa Nicolosi, Carlotta Giani, Valentina Cirello, Nicola Avenia, Stefania Rossi, Paolo Vitti, Aldo Pinchera, and Rossella Elisei



# BRAF on FNAB specimens: first studies

Non-diagnostic cytology

Histology papillary thyroid cancer




**B-RAF V600E**

Cohen et al., Clin Cancer Res 2004

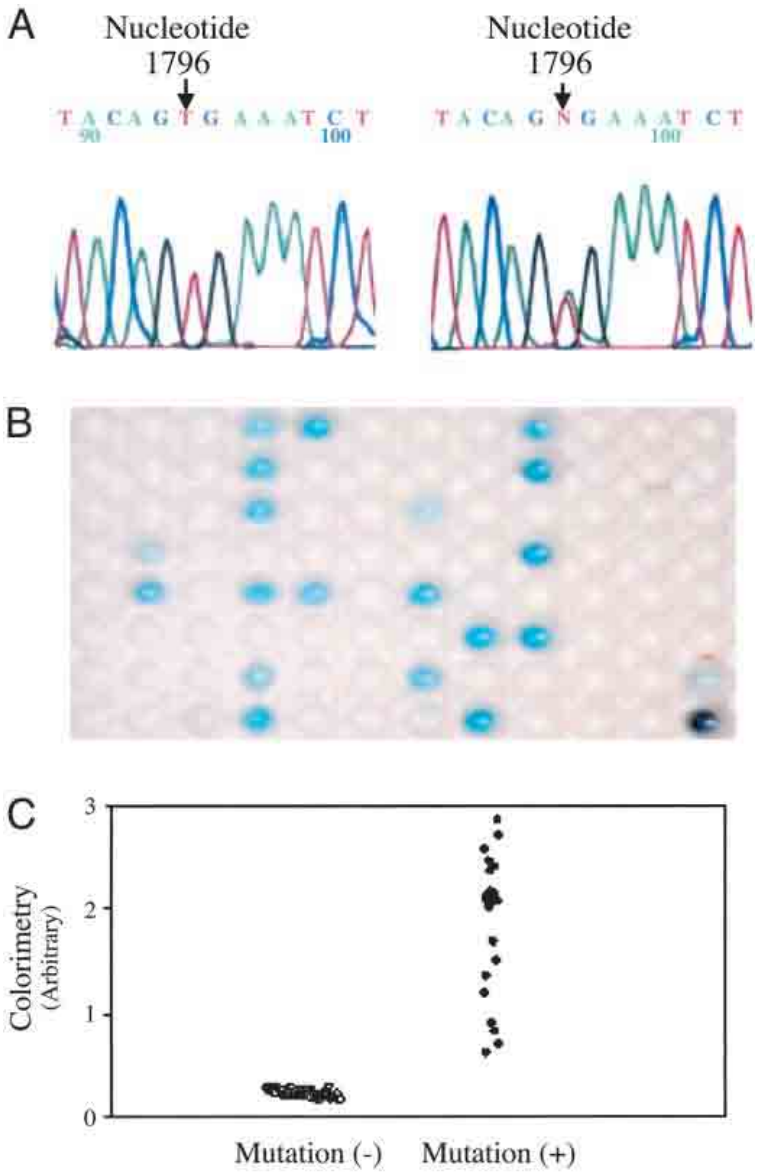



**TABLE 2.** Summary of *BRAF* mutation data on preoperative thyroid FNAB specimens (per colorimetric assay)

| Pathological diagnosis | Mutation/total | % |
|---|---|---|
| Papillary cancer | 8/16 | 50 |
| Follicular cancer | 0/5 | 0 |
| Hurthle cell carcinoma | 0/1 | 0 |
| Benign lesions | 0/21 | 0 |
| Metastatic renal cancer | 0/1 | 0 |
| Indeterminate lesion (not operated) | 0/1 | 0 |

Xing et al., 2004

**TABLE 1.** Molecular analysis of thyroid FNA

| | BRAF V599E mutation positivity | RET/PTC rearrangements[a] |
|---|---|---|
| Histology | | |
| Papillary carcinoma | 26/69 (38%) | 6/33 (18%) |
| Classic variant | 16/35 (45%) | 4/13 (30%) (3 PTC1 and 1 PTC3) |
| Follicular variant | 3/22 (14%) | 1/15 (6%) (PTC1) |
| Tall-cell variant | 5/9 (55%) | 1/5 (20%) (PTC3) |
| Diffuse-sclerosing variant | 2/3 (66%) | |
| Adenoma | 0/19 | 0/19 |
| Microfollicular | 0/5 | 0/5 |
| Micro- or macrofollicular | 0/9 | 0/9 |
| Trabecular | 0/1 | 0/1 |
| Hurthle | 0/4 | 0/4 |
| Goiter | 0/8 | 0/8 |
| Multinodular | 0/8 | 0/8 |
| Indeterminate/insufficient samples | | |
| Papillary carcinoma | 4/15 (27%) | 1/15 (7%) |
| Classic variant[b] | 2/7 (29%) | 1/7 (14%) |
| Follicular variant | 1/6 (17%) | 0/6 |
| Sclerosing variant | 1/2 (50%) | 0/2 |
| Tumor stage (PTC)[c] | | |
| T1 | 4/10 (40%) | 2/5 (40%) |
| T2 | 12/29 (41%) | 2/14 (14%) |
| T3–T4 | 7/19 (37%) | |
| Gender (PTC) | | |
| Male | 11/23 (47%) | 2/13 (15%) |
| Female | 15/46 (33%) | 4/20 (20%) |
| Age (PTC) | | |
| <40 yr | 14/37 (38%) | 2/15 (13%) |
| >40 yr | 12/32 (37%) | 4/18 (22%) |
| Node metastasis (PTC)[d] | | |
| Yes | 5/13 (38%) | 1/6 (16%) |
| No | 8/15 (53%) | 1/7 (14%) |

refinement of diagnosis in 5/15 samples

Salvatore et al., JCE&M 2004

THYROID
Volume 19, Number 11, 2009

DOI: 10.1089/thy.2009.0110



# Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer

The American Thyroid Association (ATA) Guidelines Taskforce
on Thyroid Nodules and Differentiated Thyroid Cancer

David S. Cooper, M.D.[1] (Chair)*, Gerard M. Doherty, M.D.,[2] Bryan R. Haugen, M.D.,[3]
Richard T. Kloos, M.D.,[4] Stephanie L. Lee, M.D., Ph.D.,[5] Susan J. Mandel, M.D., M.P.H.,[6]
Ernest L. Mazzaferri, M.D.,[7] Bryan McIver, M.D., Ph.D.,[8] Furio Pacini, M.D.,[9] Martin Schlumberger, M.D.,[10]
Steven I. Sherman, M.D.,[11] David L. Steward, M.D.,[12] and R. Michael Tuttle, M.D.[13]

## Indeterminate cytology

*Many molecular markers (e.g., galectin-3, cytokeratin, BRAF) have been evaluated to improve diagnostic accuracy for indeterminate nodules. Recent large prospective studies have confirmed the ability of genetic markers (BRAF, Ras, RET/PTC) and protein markers (galectin-3) to improve preoperative diagnostic accuaracy for patients with indeterminate thyroid nodules. Many of these markers are available for commercial use in reference laboratories but have not yet been widely applied in clinical practice.*

*It is likely that some combination of molecular markers will be used in the future to optimize management of patients with indeterminate cytology on FNA specimens.*

J. Endocrinol. Invest. 33 (Suppl. to no.5): 1-50, 2010

# American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association medical guidelines for clinical practice for the diagnosis and management of thyroid nodules

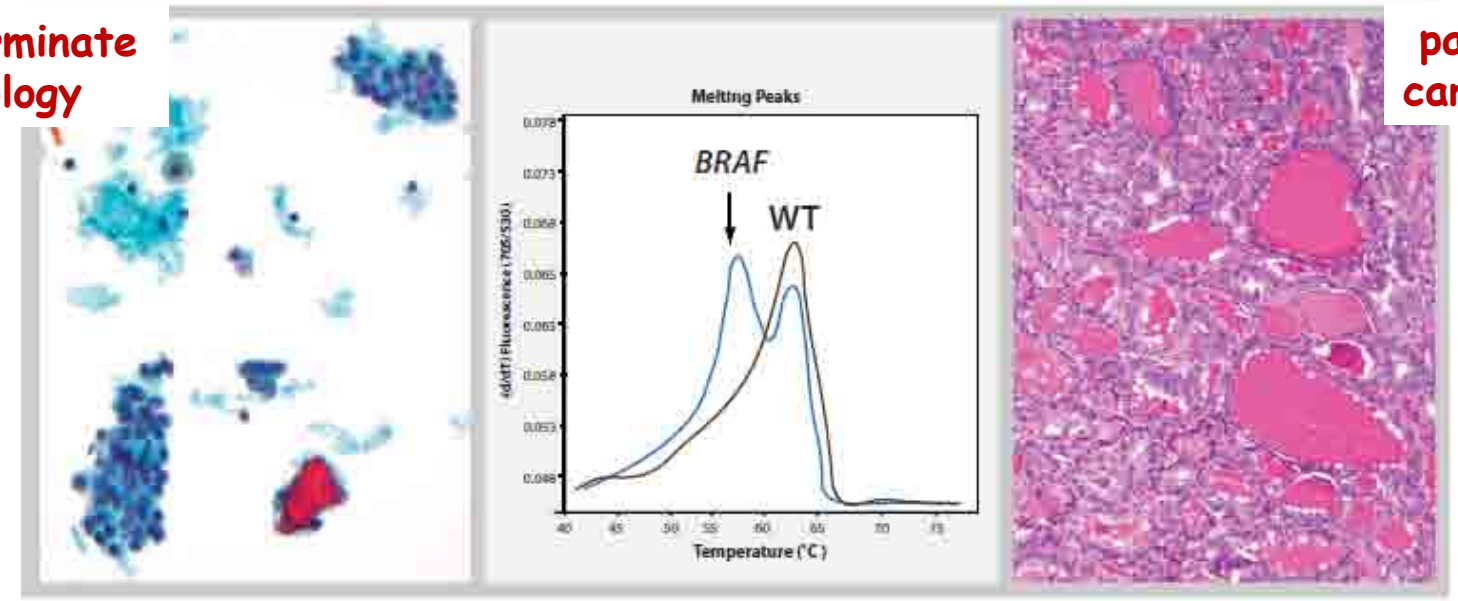
H. Gharib, E. Papini, R. Paschke, D.S. Duick, R. Valcavi, L. Hegedüs, and P. Vitti; for the AACE/AME/ETA Task Force on Thyroid Nodules*

*Task Force Committee members:* S. Tseleni Balafouta, Z. Baloch, A. Crescenzi, H. Dralle, R. Gärtner, R. Guglielmi, J.I. Mechanick, C. Reiners, I. Szabolcs, M.A. Zeiger, and M. Zini

## Indeterminate cytology

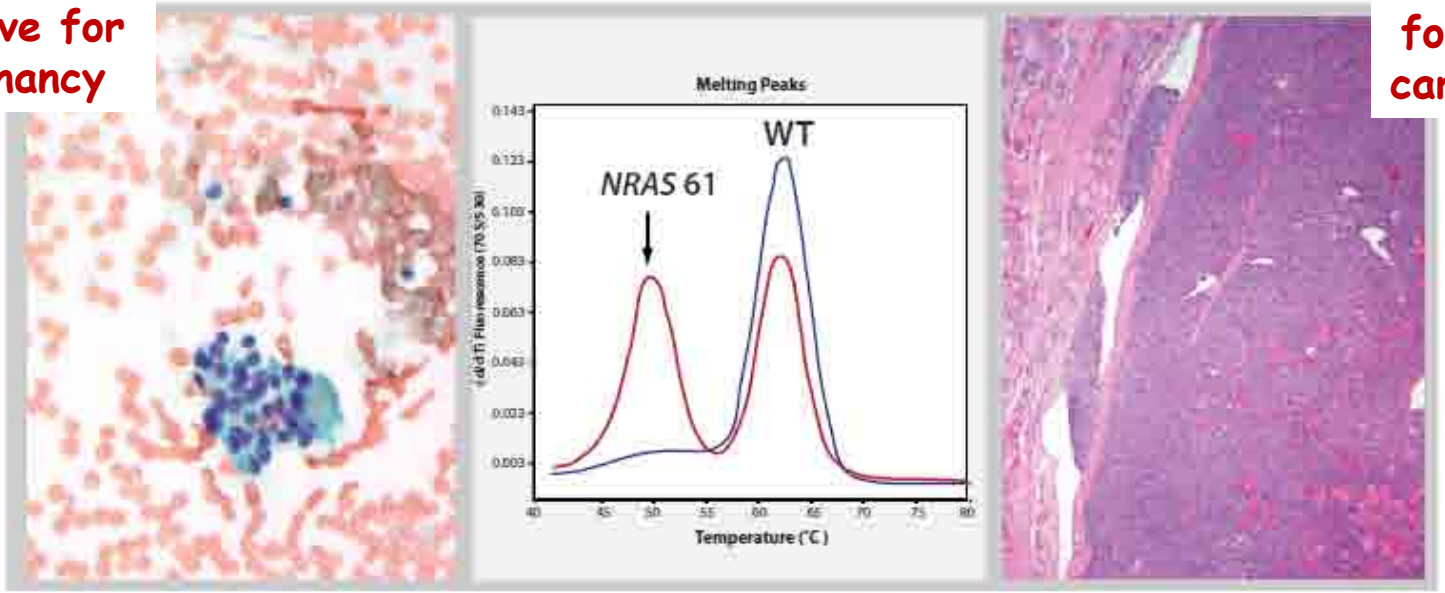
*….molecular and immunohistochemical markers may improve the accuracy of cytologic diagnosis, but they do not have consistent predictive value for malignancy and their use is still expensive and restricted to specialized centers. On the basis of current limited evidence, their routine use in clinical practice is not recommended and should be deserved for selected cases.*

indeterminate cytology




papillary carcinoma

negative for malignancy




follicular carcinoma

Nikiforov et al., JCE&M 2009

| Cytology (n=235) | Mutation (in cytology) | Histology |
|---|---|---|
| Suspicious for Thyroid cancer (n=54) | BRAF 21 | PTC 21 |
| | RET/PTC 6 | PTC 6 |
| | RAS 10 | PTC 10 |
| | None 17 | PTC 9 FA 4 Hyperplastic 4 |
| Benign (87) | BRAF 2 | PTC 2 |
| | RET/PTC 2 | PTC 2 |
| | RAS 5 | PTC 2 FA 3 |
| | None 78 | PTC 2 FTC 1 FA 10 Hyperplastic 65 |
| Indeterminate (n=41) | BRAF 2 | PTC 2 |
| | RET/PTC 2 | PTC 2 |
| | RAS 3 | PTC 2 FA 1 |
| | None 34 | PTC 1 FA 25 Hyperplastic 8 |
| Inadequate (n=53) | BRAF 8 | PTC 8 |
| | RET/PTC 1 | PTC 1 |
| | RAS 5 | PTC 2 HCC 1 FA 2 |
| | None 39 | PTC 2 FTC 2 FA 11 Hyperplastic 24 |

**41 indeterminate**
**7 PTCs at histology**
**(2 BRAF, 2 RET/PTC, 2 RAS)**

**mutations in 28.5% cytological samples (235 nodules)**

34.3% ras

49.3% Braf

16.4% ret/PTC

**cancer in 74% histological samples**

**cancer in 100% histological samples**

Cantara et al., 2010

# High diagnostic performance of combined cytology and molecular analysis

**TABLE 1.** Diagnostic performance of cytology, molecular analysis, or a combination of both

| Diagnostic modality | Sensivity TP/TP+FN (%) | Specificity TN/FP+TN (%) | PPV TP/TP+FP (%) | NPV TN/TN+FN (%) | Accuracy TP+TN/All (%) |
|---|---|---|---|---|---|
| Cytology (positive for malignancy) | 59.0 | 94.9 | 85.2 | 82.3 | 83.0 |
| Molecular analysis (mutation in malignancy)[a] | 78.2 | 96.2 | 91.0 | 89.9 | 90.2 |
| Molecular analysis (mutation in malignancy)[b] | 79.8 | 100 | 100 | 89.9 | 92.8 |
| Cytology and molecular analysis[a] | 89.7 | 94.9 | 89.7 | 94.9 | 93.2 |
| Cytology and molecular analysis[b] | 90.5 | 98.7 | 97.4 | 94.9 | 95.7 |

TP, True positive; TN, true negative; FN, false negative; FP, false positive.

[a] Mutated follicular adenomas computed as false positive.

[b] Mutated follicular adenomas computed as true positive.

Cantara et al., 2010

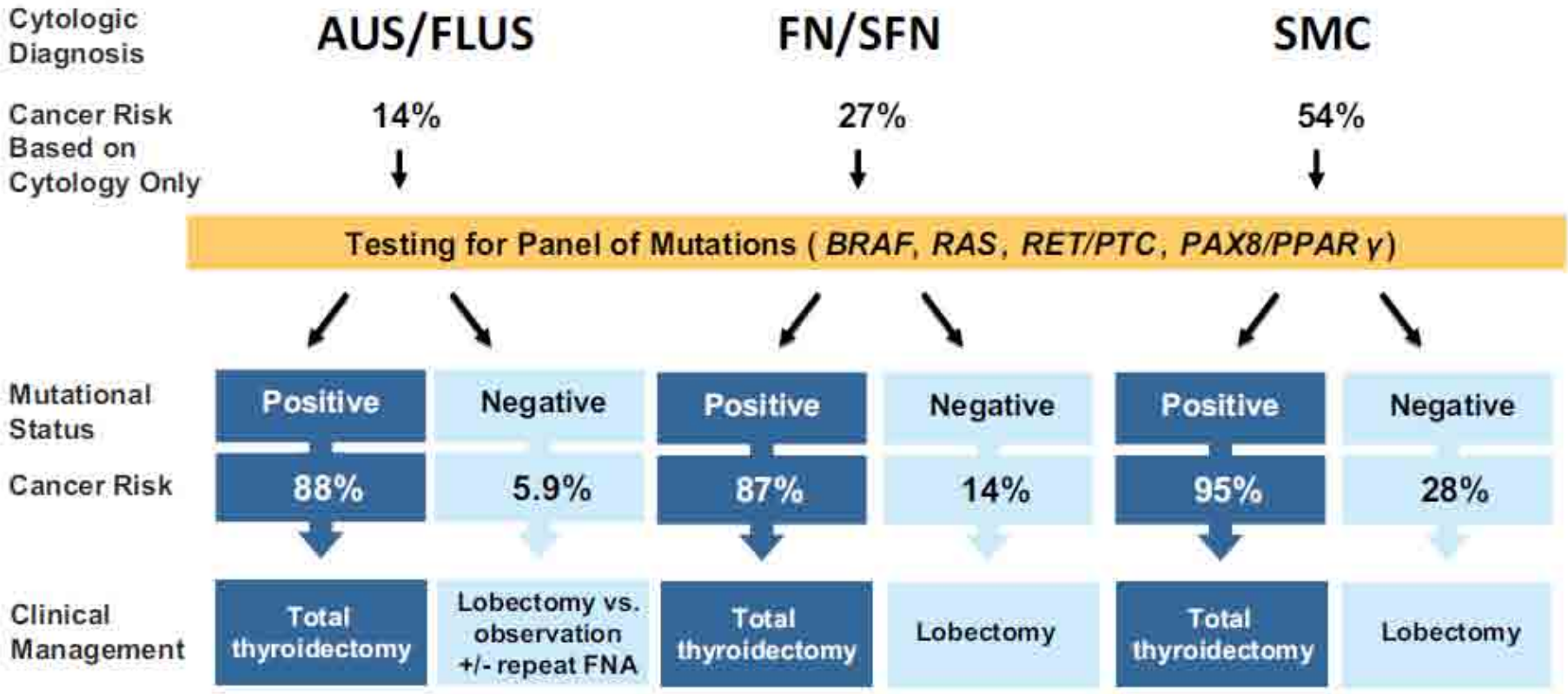
| Atypia of undetermined significance/Follicular lesions of undetermined significance (AUS/FLUS) (n=247) | | | |
|---|---|---|---|
| | **Histology Malignant (n=35)** | **Histology Benign (n=212)** | |
| **Mutation Positive (n=25)** | 16 *RAS* (16 PTC,FV)<br>5 *BRAF* (4 PTC, 1 PTC,FV)<br>1 *PAX8/PPARg* (1 PTC,FV) | 3 *RAS* (3 FA) | ***Sensitivity*** **63%**<br>***Specificity*** **99%**<br>***PPV*** **88%**<br>***NPV*** **94%**<br>***Accuracy*** **94%** |
| **Mutation Negative (n=222)** | 13 (11 PTC, FV, 2 PTC) | 209 (166 HN, 43 FA) | |

| Follicular or Hürthle cell neoplasm/Suspicious for follicular neoplasm (FN/SFN) (n=214) | | | |
|---|---|---|---|
| | **Histology Malignant (n=58)** | **Histology Benign (n=156)** | |
| **Mutation Positive (n=38)** | 2 *BRAF* (1 PTC, 1 PTC,FV)<br>29 *RAS* (21 PTC,FV, 5 PTC, 3 FTC)<br>2 *PAX8/PPARg* (2 PTC,FV) | 5 *RAS* (5 FA) | ***Sensitivity*** **57%**<br>***Specificity*** **97%**<br>***PPV*** **87%**<br>***NPV*** **86%**<br>***Accuracy*** **86%** |
| **Mutation Negative (n=176)** | 25 (16 PTC,FV, 3 PTC, 6 FTC) | 151 (95 HN, 56 FA) | |

| Suspicious for malignant cells (SMC) (n=52) | | | |
|---|---|---|---|
| | **Histology Malignant (n=28)** | **Histology Benign (n=24)** | |
| **Mutation Positive (n=20)** | 10 *BRAF* (10 PTC)<br>7 *RAS* (6 PTC,FV, 1 FTC)<br>1 *PAX8/PPARg* (1 FTC)<br>1 *RET/PTC* (1 PTC) | 1 *RAS* (1 FA) | ***Sensitivity*** **68%**<br>***Specificity*** **96%**<br>***PPV*** **95%**<br>***NPV*** **72%**<br>***Accuracy*** **81%** |
| **Mutation Negative (n=32)** | 9 (7 PTC, 2 PTC,FV) | 23 (17 HN, 6 FA) | |

Nikiforov et al., 2011

# clinical algorithm for management of patients with cytologically indeterminate thyroid FNA applying the results of mutational analysis




Nikiforov et al., JCE&M 2011

Asuragen

# The Asuragen miRInform Molecular Panel

**miR*Inform*™ Thyroid Panel**

| DNA Mutation Markers | | | | RNA fusion transcripts |
|---|---|---|---|---|
| KRAS | BRAF | HRAS | NRAS | RET/PTC1 |
| G12R | V600E | Q61L | Q61R | RET/PTC3 |
| G12V | | Q61R | Q61K | PAX8/PPARg |
| G13D | | G12V | Q61L | |
| G12D | | | | |
| G12A | | | | |
| G12C | | | | |
| G12S | | | | |

## Instructions for FNA collection in RNA*Retain*™*

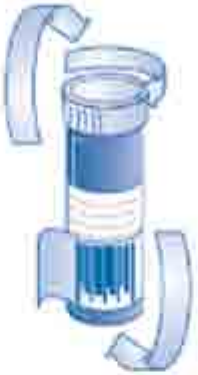
1


Remove small RNA*Retain*™* vial from kit and unscrew top.

2


Upon collection, immediately add FNA biopsy to vial, washing the needle only, 1-2 times.

3



Screw top back on vial and add bar-coded label. Invert 2-3 times.

4


Place specimen vial in styrofoam insert and seal specimen collection bag around the styrofoam and vial. Place sealed sample bag back into kit.

5

Fold and place completed requisition form inside box but, outside of specimen collection bag.

6


Insert specimen box into FedEx Clinical Pak, adhere shipping label and ship.

*For research use only. Not for use in diagnostic procedures.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

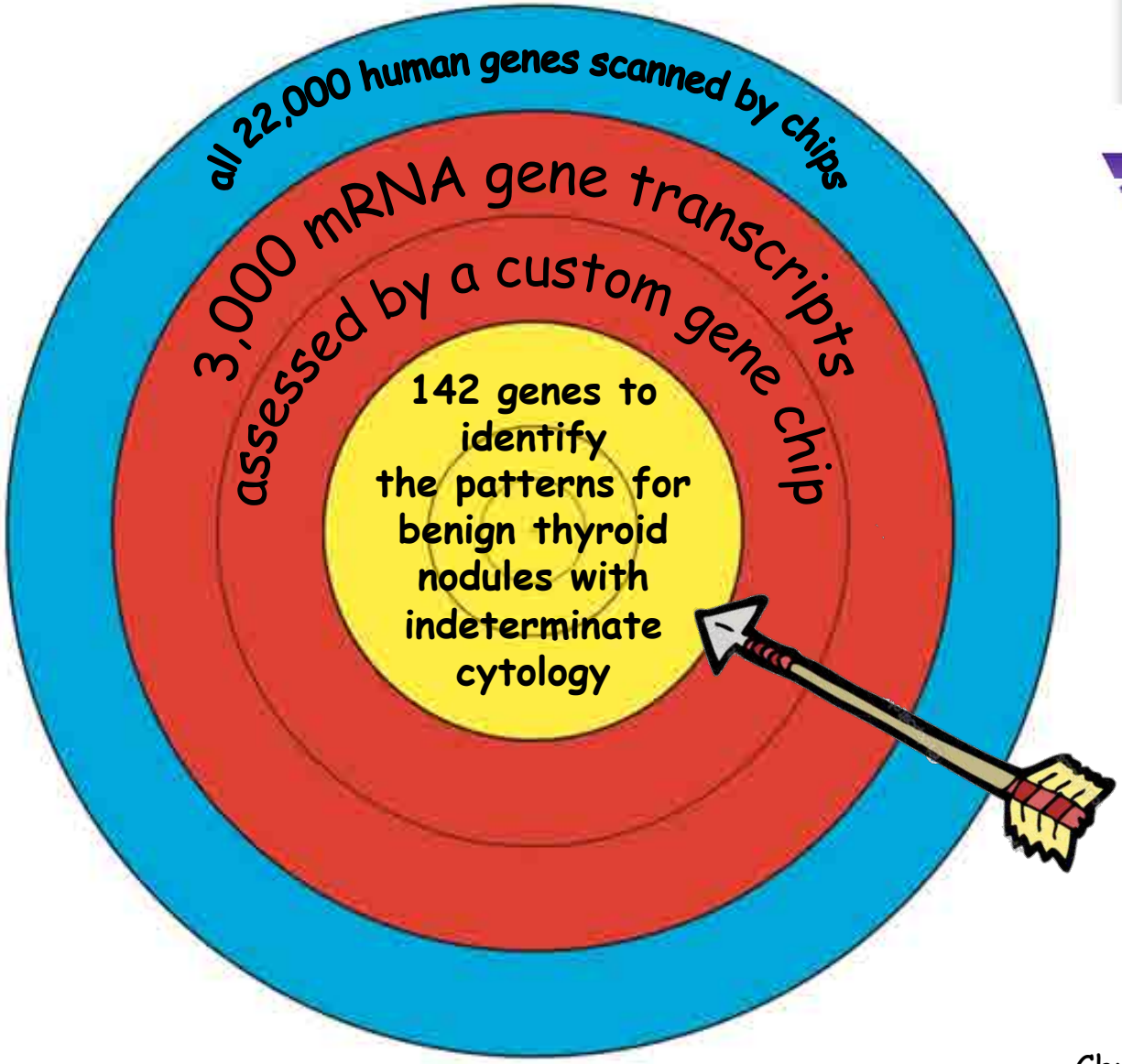
# Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology

Erik K. Alexander, M.D., Giulia C. Kennedy, Ph.D., Zubair W. Baloch, M.D., Ph.D., Edmund S. Cibas, M.D., Darya Chudova, Ph.D., James Diggans, Ph.D., Lyssa Friedman, R.N., M.P.A., Richard T. Kloos, M.D., Virginia A. LiVolsi, M.D., Susan J. Mandel, M.D., M.P.H., Stephen S. Raab, M.D., Juan Rosai, M.D., David L. Steward, M.D., P. Sean Walsh, M.P.H., Jonathan I. Wilde, Ph.D., Martha A. Zeiger, M.D., Richard B. Lanman, M.D., and Bryan R. Haugen, M.D.

Alexander et al., NEJM 2012

# The gene expression classifier

Afirma
Thyroid FNA Analysis




Chudova et al., 2010

# multiple steps and multiple days to run the Afirma Gene Expression Classifier

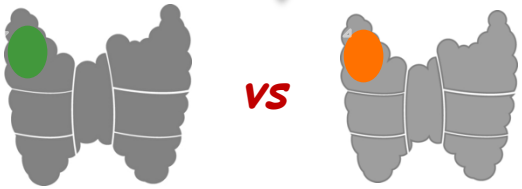
Accession Patient Sample and Requisition Form

DAY 1 — Extract, purify and measure RNA

DAY 2, 3 — Amplify RNA via cDNA

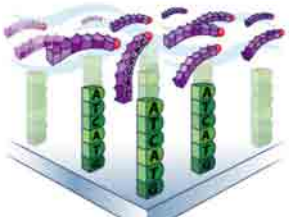
DAY 4 — Run on Afirma custom chip

Run software and generate result



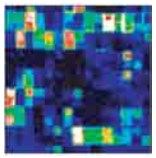

**Benign, Suspicious or No Result**


Gene chip



Sample cDNA fragments washed over DNA probe array

Corresponding fragments bind



Intensity of each gene is measured to determine expression level

Chudova et al, *JCE&M* 2010; Image Source: Affymetrix®

**Performance across the Primary Data Set of Indeterminate Nodules (N=265)**

| GEC result | Malignant reference standard (N=85) | Benign reference standard (N=180) |
|---|---|---|
| Suspicious | 78 | 87 |
| Benign | 7 | 93 |

Sensitivity, 92% (84–97); specificity, 52% (44–59); PPV, 47% (40–55); NPV, 93% (86–97); prevalence of malignant lesions, 32%

**Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (N=129, 48.7%)**

| GEC result | Malignant reference standard (N=31) | Benign reference standard (N=98) |
|---|---|---|
| Suspicious | 28 | 46 |
| Benign | 3 | 52 |

Sensitivity, 90% (74–98); specificity, 53% (43–63); PPV, 38% (27–50); NPV, 95% (85–99); prevalence of malignant lesions, 24%

**Follicular or Hürthle-Cell Neoplasm or Suspicious for Follicular Neoplasm (N=81, 30.6%)**

| GEC result | Malignant reference standard (N=20) | Benign reference standard (N=61) |
|---|---|---|
| Suspicious | 18 | 31 |
| Benign | 2 | 30 |

Sensitivity, 90% (68–99); specificity, 49% (36–62); PPV, 37% (23–52); NPV, 94% (79–99); prevalence of malignant lesions, 25%

**Suspicious for Malignancy (N=55, 20.8%)**

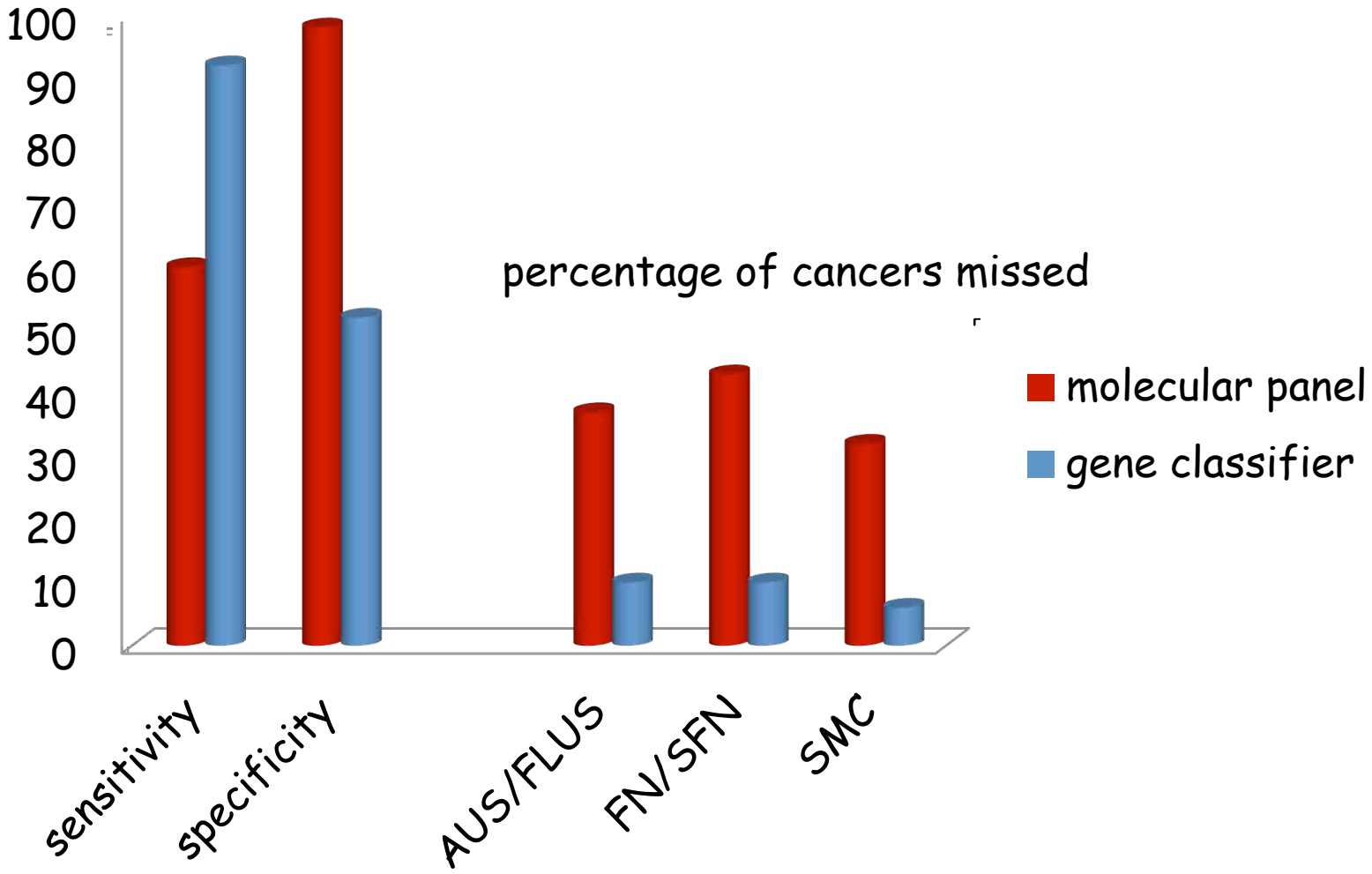
| GEC result | Malignant reference standard (N=34) | Benign reference standard (N=21) |
|---|---|---|
| Suspicious | 32 | 10 |
| Benign | 2 | 11 |

Sensitivity, 94% (80–99); specificity, 52% (30–74); PPV, 76% (61–88); NPV, 85% (55–98); prevalence of malignant lesions, 62%

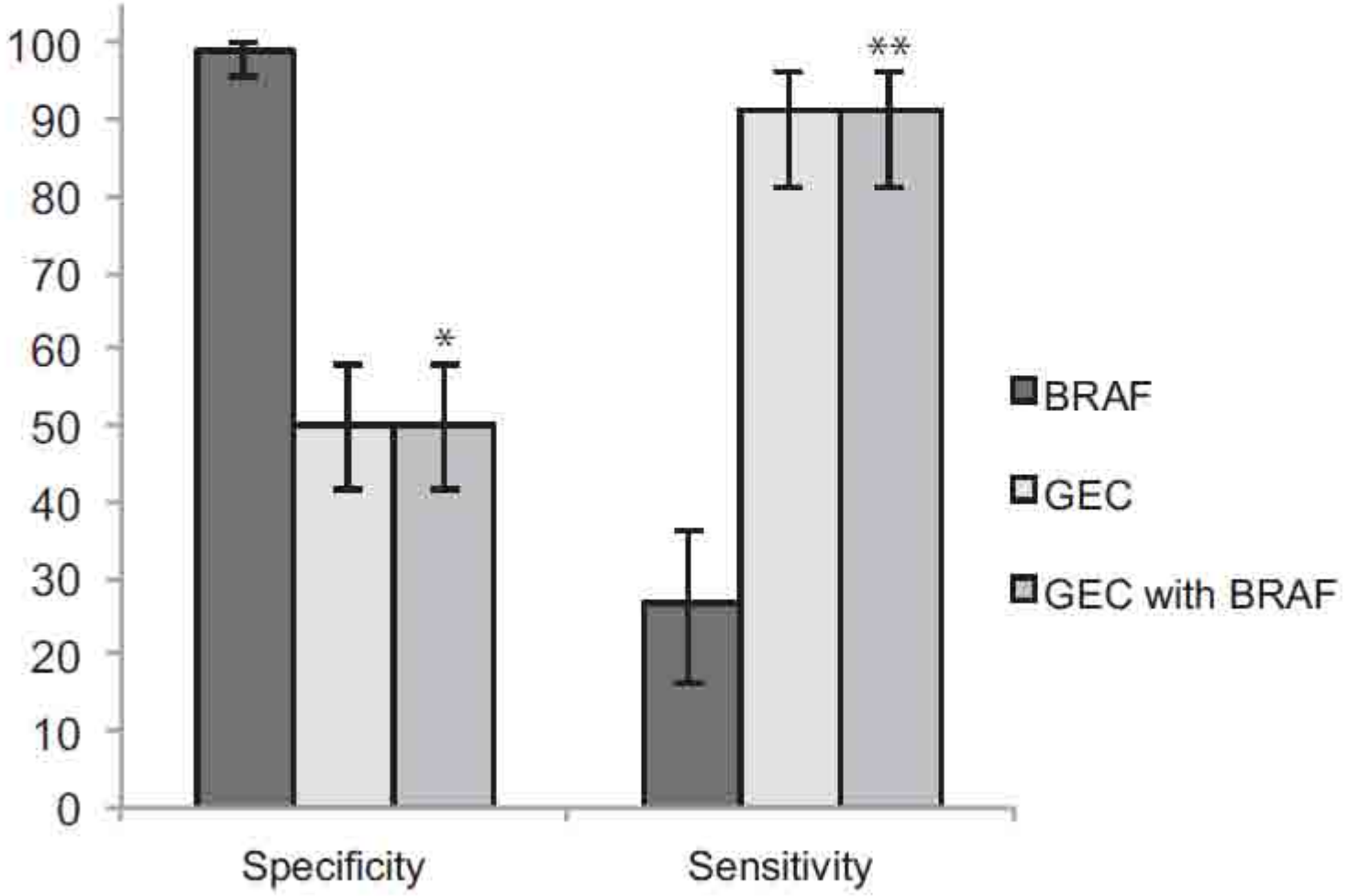
# Performance of the Gene-Expression Classifier according to the final histopathological diagnoses for cytologically indeterminate samples

Alexander et al., NEJM 2012

# molecular panel *vs* gene classifier



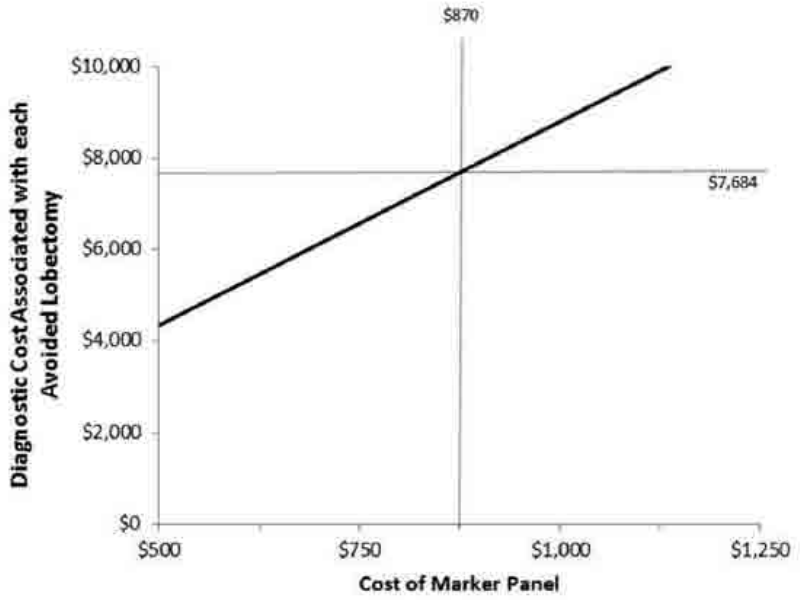
# Neither GEC test sensitivity nor specificity is improved by addition of BRAF mutation testing




Kloos et al., 2013

**Mutations in 15-25% of FNA specimens**
with a very low frequency
in atypia/FLUS and
Follicular Neoplasm sub-types

## In sensitivity analysis, savings were demonstrated if molecular testing cost was less than $870




**Cost of Asuragen miRInform Thyroid panel $2250**

Medicare reimbursement for this test is currently $650, while the range of reimbursements from private insurers varies up to $950

**cost savings with molecular testing of FNA results in two indeterminate cytological categories: FLUS and FN**

Yip et al., JCEM 2012

# GEC in patients with indeterminate thyroid nodules
# overall costs reduction
# quality of life improvement

Treatment with current standard of care practice

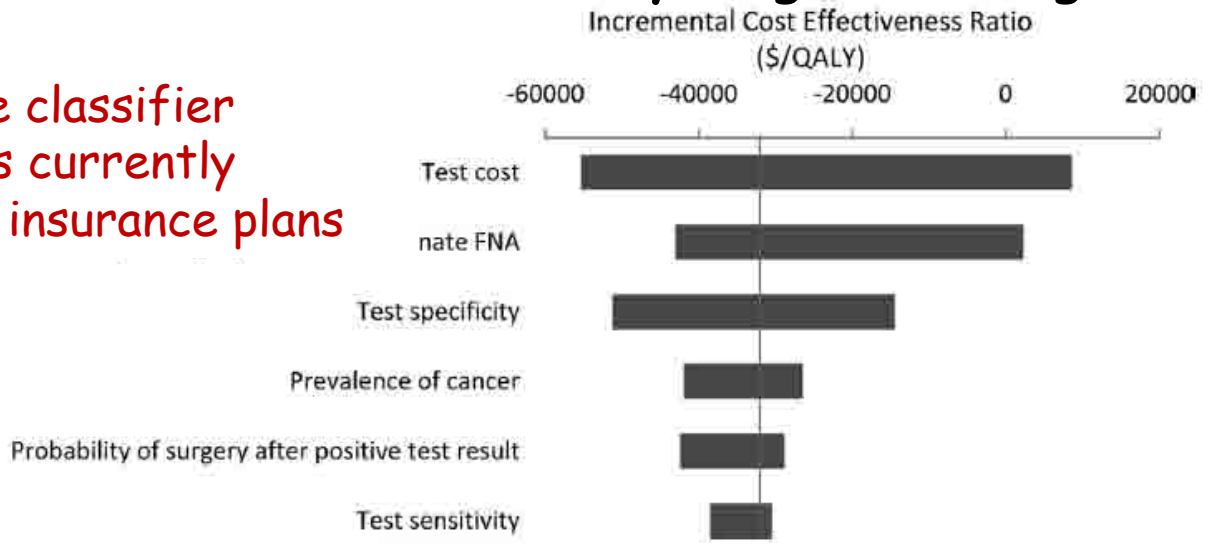
without molecular testing = $12,172/pt

Treatment with current standard of care practice

with GEC test = $10,719/pt

**(3/4 reduction in the number of unnecessary diagnostic surgeries)**

the Afirma gene classifier
(3200 $/test) is currently
not covered by some insurance plans



# Cytology and BRAF determination why not?

**Table 2.** Distribution of *BRAF*-Positive Cases Among Indeterminate and Malignant Diagnoses[a]

| Study | BSRTC | AUS/FLUS | FN/SFN | SMC | PMC | Type of *BRAF* Mutation Detected |
|---|---|---|---|---|---|---|
| Marchetti 2009[23] | No | — | 0/59 (0%) | 18/59 (30.5%) | 41/59 (69.5%) | V600E |
| **Nikiforov 2009[12]** | **Yes** | **1/19 (5.2%)** | **4/19 (21.1%)** | **4/19 (21.1%)** | **10/19 (52.6%)** | **V600E** |
| **Zatelli 2009[24]** | **Yes** | **0/42 (0%)** | **1/42 (2.4%)** | **10/42 (23.8%)** | **31/42 (73.8%)** | |
| Kim 2010[25] | No | — | 0/212 (0%) | 50/212 (23.6%) | | |
| Moses 2010[26] | No | 3/23 (13.0%) | | | | |
| **Proietti 2010[27]** | **Yes** | **4/26 (15.4%)** | | | | |
| **Adeniran 2011[28]** | **Yes** | | | | | |
| Colanta 2011[29] | No | | | | | |
| **Kim 2011[31]** | | | | | | |
| Pelizzo 20 | | | | | | V600E |
| Chang 201 | | | | | 57/92 (62.0%) | V600E |
| **Kang 2012** | | | | **76/276 (27.5%)** | **200/276 (72.5%)** | **V600E** |
| Marchetti 2 | | | 0/63 (0%) | 32/63 (50.8%) | 31/63 (49.2%) | V600E |
| **Current stu** | | **11/119 (9.2%)** | **4/119 (3.4%)** | **14/119 (11.8%)** | **90/119 (75.6%)** | **V600E/K601E/other** |

**(0-20%)** **(0-31%)**

**Improvement in cytologic classification**

Ohori et al., 2013

# Molecular analysis of FNAB material: clinical implications

**Table 2.** Association of *BRAF* Mutation Status Detected on Thyroid Fine-Needle Aspiration Biopsy With Persistence/Recurrence of Papillary Thyroid Cancer

| Characteristic | *BRAF* Positive No. | *BRAF* Positive % | *BRAF* Negative No. | *BRAF* Negative % | *P** |
|---|---|---|---|---|---|
| All types of PTC | | | | | |
| No. of patients | 53 | | 76 | | |
| Recurrence/persistence | 19 | 35.8 | 9 | 11.8 | .002 |
| $^{131}$I dose, mCi | | | | | .01 |
| Median | 100 | | 79 | | |
| Range | 0-211 | | | | |
| Follow-up, month | | | → | 8.3 | .004 |
| | | | | | .014 |
| | 100 | | 77 | | |
| Range | 0-209 | | 0-204 | | |
| Follow-up, months | | | | | .75 |
| Median | 26 | | 29 | | |
| Range | 7-114 | | 7-116 | | |
| Follow-up, months† | | | | | .32 |
| Median | 36 | | 31 | | |
| Range | 9-114 | | 7-116 | | |

preoperative identification of patients at risk for extensive disease

more aggressive treatment?

Xing et al., J Clin Oncol 2009

# Preoperative BRAF analysis facilitate prediction of occult VI level metastatic lymph-nodes

**TABLE 3.** Association of *BRAF* mutation detected on thyroid FNAB with clinicopathological characteristics of 148 PTC patients

| Variables | Positive *BRAF* mutation (n = 79) | Negative *BRAF* mutation (n = 69) | *P* value |
|---|---|---|---|
| Mean age at diagnosis [yr (range)] | 51.5 (28–81) | 53.5 (26–73) | |
| Gender, male [n (%)] | 10 (12.7) | [illegible] | |
| Mean tumor size [mm (range)] | 9.3 (3.0–40.0) | [illegible] | |
| Multifocality [n (%)] | [illegible] | [illegible] | |
| Perithyroidal invasion [n (%)] | [illegible] | [illegible] | |
| Lymphovascular [illegible] | [illegible] | [illegible] | |
| [illegible] | [illegible] | [illegible] | 0.198 |
| [illegible] | [illegible] | 56 (81.2) | |
| [illegible] | [illegible] | 13 (18.8) | |

[illegible] for a given variable.

**preoperative identification of patients at risk for lymph nodal involvement**

prophylactic central neck dissection?

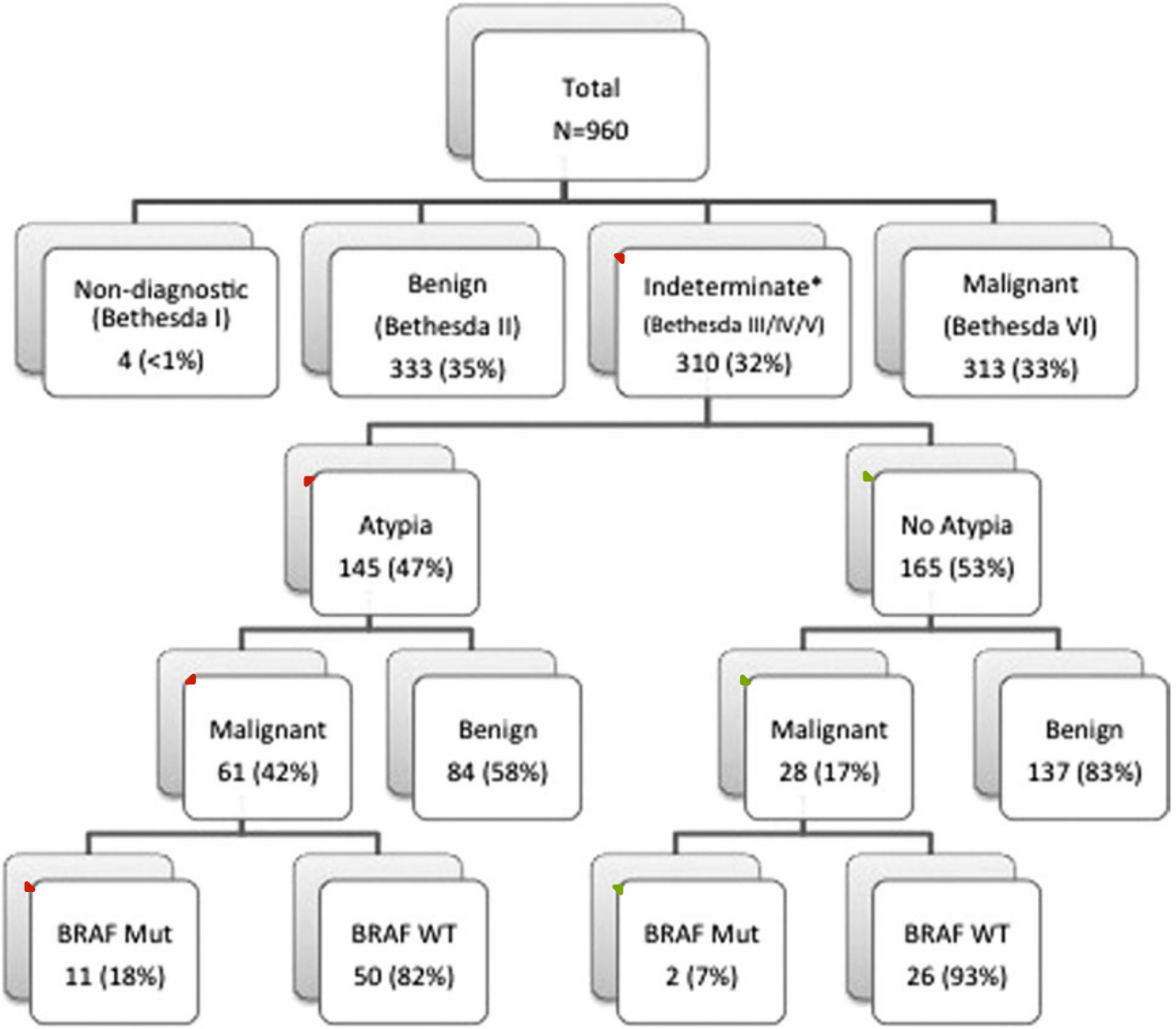
Joo et al., JCE&M 2012

# Back to cytology alone?

BRAF $^{V600E}$ mutation is most common in nodules with other cytologic risk factors for malignancy, which already warrant a total thyroidectomy. Therefore, single-mutation screening for BRAF $^{V600E}$ does not meaningfully improve preoperative risk stratification

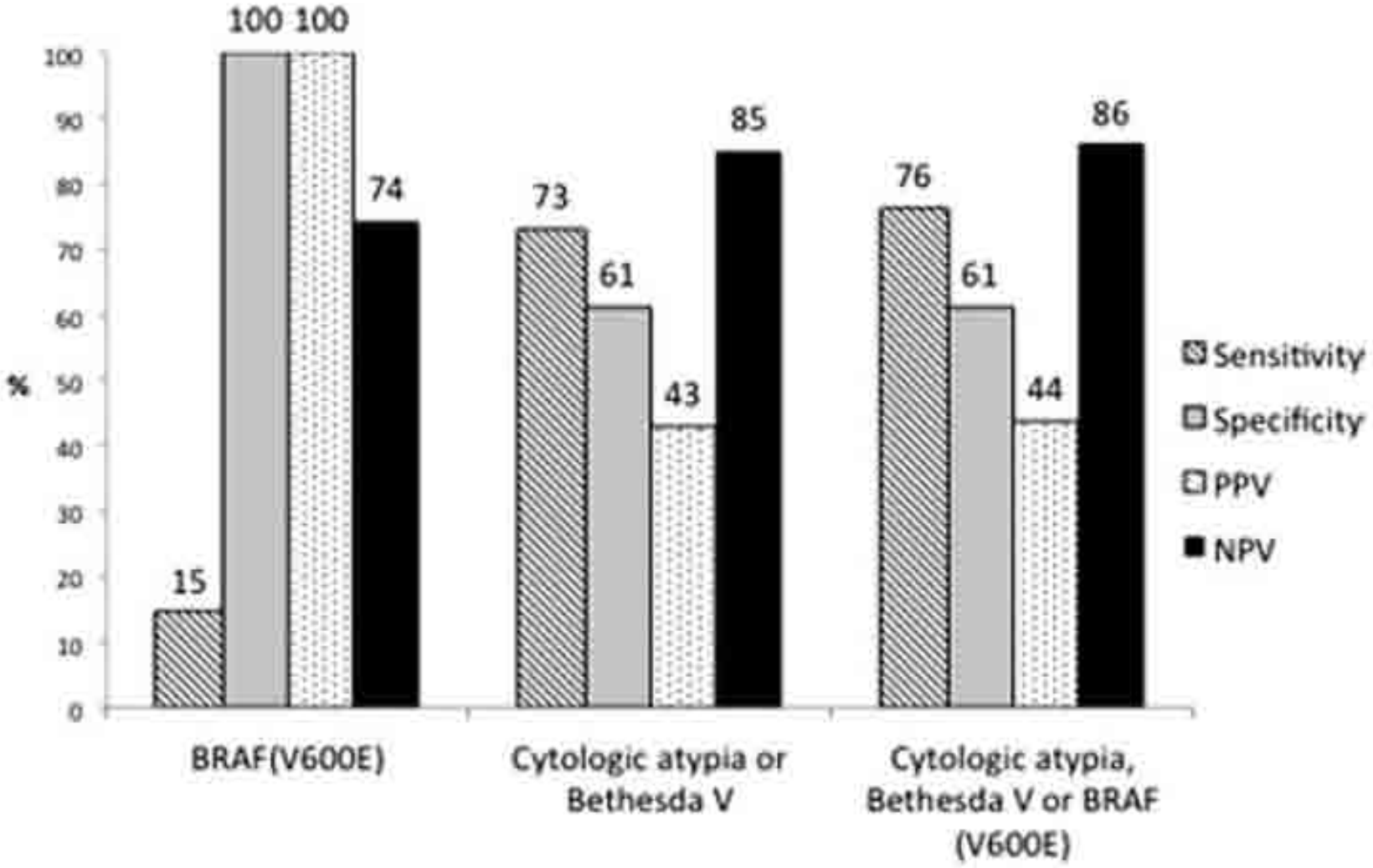
Kleiman et al., Cancer 2013

# BRAF(V600E) mutation is most common in nodules with other cytologic risk factors for malignancy




Kleiman et al., 2013

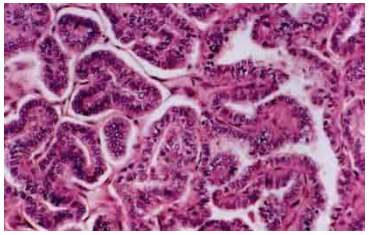
**……no meaningful improvement in sensitivity, specificity, NPV, or PPV when BRAF testing is added to conventional cytology**




Kleiman et al, 2013

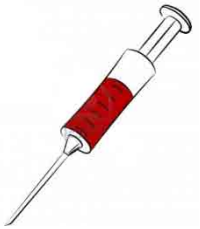
# The evolution in thyroid cancer diagnosis

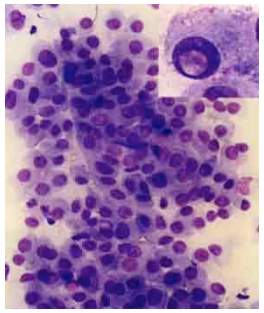
**Surgery** → **Needle Aspirate Cytology** → **Molecular Cytology**



**Histopathology**

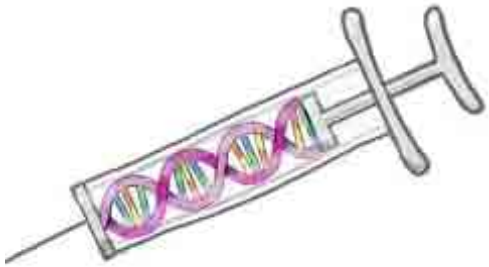
**1600's**





**FNA Cytology**
Reduced unnecessary surgery, except when **indeterminate**

**1960's - 1980's**



**FNA Molecular**
Refinement of cytology to reduce unnecessary diagnostic surgery or to tailor treatment

**2012**

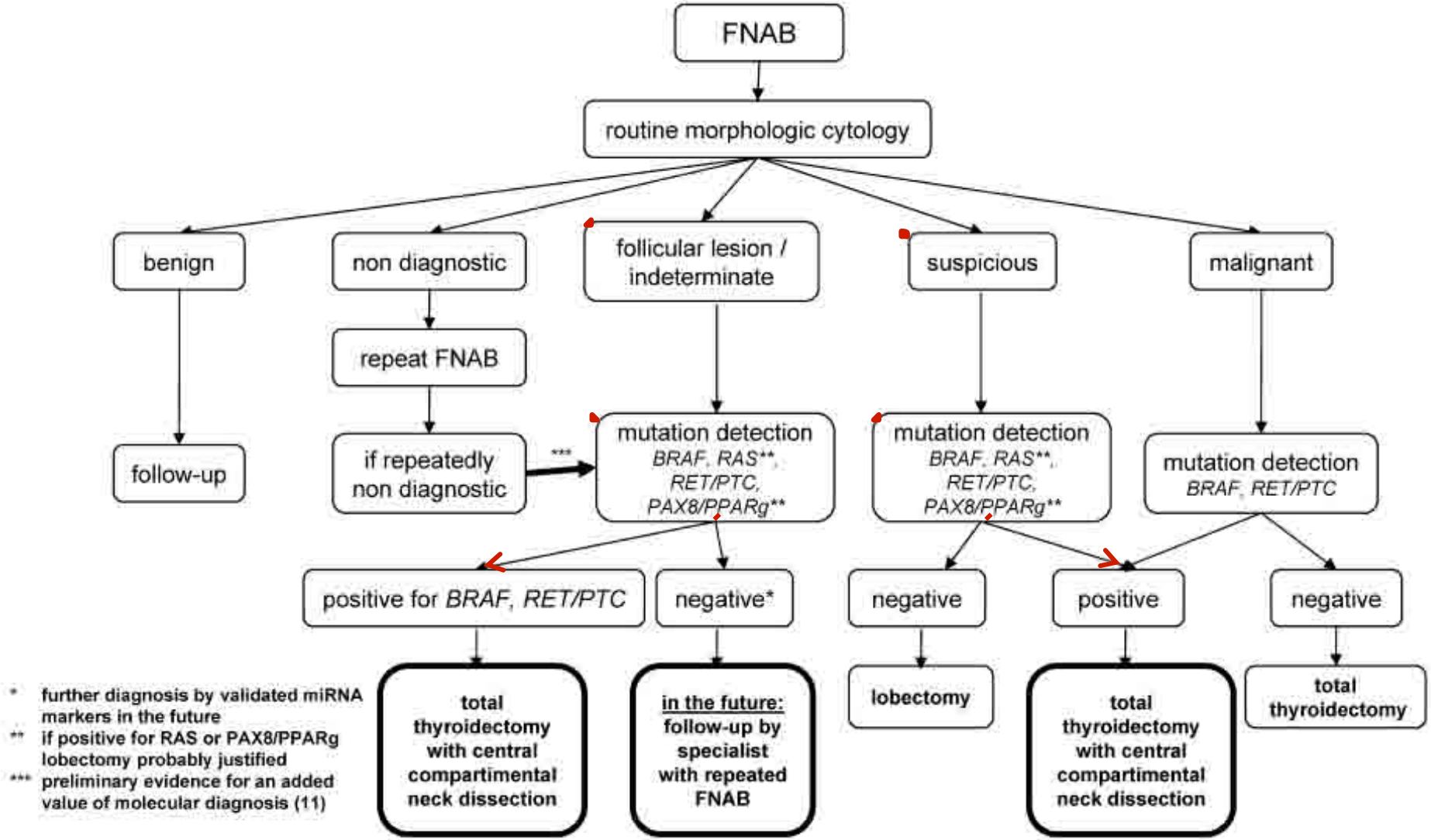
# The goals of molecular FNAB

**TABLE 2.** Current classification of thyroid FNAB by different organizations and the respective goals for molecular FNAB

| AACE/AME/ ETA, 2010 (1) | BTA, 2007 (3) | ATA, 2009 (5) | NCI, 2008 (83) | Molecular FNAB goals |
|---|---|---|---|---|
| Nondiagnostic | Nondiagnostic | Nondiagnostic/ inadequate | Nondiagnostic/unsatisfactory | 26% Mutation positive (39). Reduce rate of a second FNAB |
| Benign | Benign | Nonneoplastic | Benign | Reduce the FN rate in settings with high FN between 6 and 17% (9) |
| Follicular lesion | Follicular lesion | Indeterminate | Follicular lesion of undetermined significance/ atypia of undetermined significance | Improve the differential diagnosis between benign and malignant. Sensitivity 85.7/97%, specificity 97/ 100% (39, 40). Reduce the rate of diagnostic surgery. Increase the rate of total thyroidectomy as first surgery |
| Suspicious | Suspicious | Suspicious (for PTC) | Follicular-neoplasm/suspicious for follicular neoplasm Hurthle cell neoplasm. Suspicious for malignancy | Improve the differential diagnosis between benign and malignant. Increase the rate of total thyroidectomy as first surgery (53) |
| Malignant | Malignant | Malignant | Malignant | Increase the rate of total thyroidectomy as first surgery and define the extension of the surgery |

Ferraz……Paschke, JCE&M 2011

# diagnostic algorithms are changing




Ferraz…….Paschke, JCE&M 2011

THYROID
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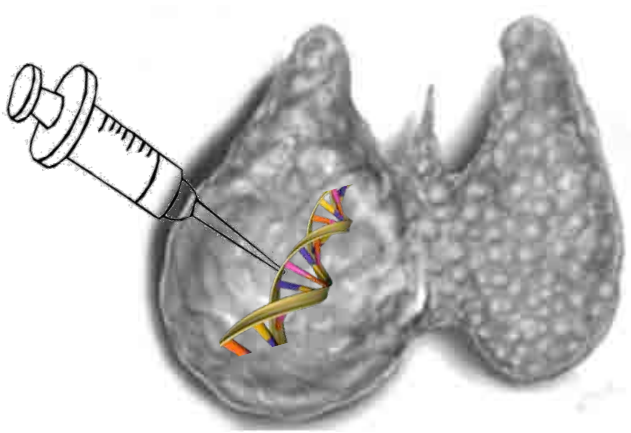
# Information for Clinicians: Commercially Available Molecular Diagnosis Testing in the Evaluation of Thyroid Nodule Fine-Needle Aspiration Specimens

Steven P. Hodak[1] and David S. Rosenthal[2]

for the American Thyroid Association Clinical Affairs Committee

TSH receptor mRNA reverse transcription–polymerase chain reaction, the Veracyte and Asuragen commercial methods, and the noncommercial use of *BRAF*, *RAS*, *RET/PTC*, and *PAX8/PPARγ* testing have promising roles in the diagnosis and treatment of patients with nodular thyroid disease and thyroid cancer. However, at this time, experience with these molecular methods remains limited, and no test has perfect sensitivity and specificity. Peer-reviewed data evaluating the diagnostic performance of these tests are increasingly available. The American Thyroid Association (ATA) feels that until an expert consensus review of existing data (now underway by the ATA Guidelines Task Force) can be completed, no evidence-based recommendation for or against the use of these methods can be made. Clinicians are therefore advised to consider the use of these genetic diagnosis methods with appropriate caution, and to remain cognizant of the limitations of the data supporting their use. Patients who are interested in the use of these tests in their own care should discuss them thoroughly with their care providers. Until evidence-based recommendations are available, determining whether or not the limited data available support the use of these methods should be considered on a case-by-case basis.



# Thank you for your attention

laura.fugazzola@unimi.it

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